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COMPARATIVE EVALUATION OF *FICUS EXASPERATA* VAHL. AQUEOUS LEAF EXTRACT AND SPIRONOLACTONE ON URINARY EXCRETIONS IN NORMOTENSIVE RATS

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ABSTRACT

Medicinal plants known diuretics are commonly used to treat hypertension and edema. *Ficus exasperata* aqueous leaf extract (FEFIX) lowers blood pressure in a dose-dependent. This extract had a relatively large and higher diuretic and electrolyte effect than that induced by a loop diuretic. The effectiveness of diuretics was based on their site of action in the nephron. The aim of this work was to compare the effects of FEFIX to those of spironolactone (SPLT), aldosterone antagonist, in normal rats. Single doses of FEFIX (100 mg/kg b.w.) and SPLT (10 mg/kg b.w.) were orally administered to two groups of rats. A control group received NaCl (9 ‰). The urine was collected and sampled every two hours for 24 hours. Blood was drawn at the end of the experiment. Levels of electrolytes (Na, K, Cl and Ca 2), urea and creatinine were measured in urine and serum. FEFIX and SPLT increased urine volume and urinary excretion of sodium and chlorine. Both substances studied decreased serum sodium and serum chloride. FEFIX increased the urinary excretion of creatinine and urea without significantly altering their plasma concentration. The conclusion

of this work is that the aqueous extract of *F. exasperata* leaves had a relatively high diuretic activity. This extract changed the plasma levels of electrolytes like SPLT after 24 hours.

Key words: Urinary excretion, Electrolytes, *Ficus exasperata*, Spironolactone.

INTRODUCTION

Medicinal plants were commonly used in primary health care. They can treat many diseases [1-2]. Plant species known diuretics were commonly used in cases of hypertension and edema [3]. Among these species was shown *Ficus exasperata* (Moraceae). The aqueous leaf extract lowers blood pressure in a dose-dependent. This effect was reduced in the presence of atropine [4-5]. In addition, this extract increased urine volume and urinary excretion of electrolytes. The diuretic effect induced by this extract was relatively large and higher than that induced by furosemide, a loop diuretic [6]. The use of diuretics is to increase the volume of urine excreted. However diuretics acted at different levels of the nephron and were classified into different groups. Their site of action was closely related to their effectiveness [7]. The treatment of certain forms of hypertension and edema in the case of kidney failure required a class of diuretics or the combination of different types of diuretics [8-9]. Spironolactone, aldosterone antagonist promoted sodium excretion by interacting directly with the mineralocorticoid receptor in the distal tubule and collecting duct [10-11]. In addition, this diuretic inhibited the action of aldosterone on the activity of Na-K ATPase in the cortical collecting tubule [12]. The aim of this study was to compare the effect of an aqueous extract of leaves of *F. exasperata* (FEFIX) and that of spironolactone, diuretic aldosterone antagonist, on urinary excretion.

MATERIAL AND METHODS

Ethics

Experimental procedures and protocols used in this study were approved by Ethical Committee of Health Sciences, University Felix Houphouet-Boigny. These guidelines were in accordance with the internationally accepted principles for laboratory animal use and care [13-14].

Plant material

Fresh leaves of *F. exasperata* Vahl. 1805 (Moraceae) were collected in a forest of the Southern region of Côte d'Ivoire (Region des Lagunes). This plant was authenticated by a

Botany expert, Prof. Ake-Assi Laurent of the "Centre National de Floristique", UFR-Biosciences, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire.

Chemicals used

Spironolactone used for this study was obtained from Pfizer Holding (France). Physiological saline solutions were prepared from sodium chloride from Sigma-Aldrich (France).

Preparation of *Ficus exasperata* aqueous leaf extract

Ficus exasperata aqueous leaf extract (FEFIX) preparation was previously described [5]. Fresh leaves of *F. exasperata* were washed and dried in an oven at a temperature of 40 ± 2 ° C. They were pulverized to obtain a fine powder which was left to macerate in *n*-hexane at a rate of 10 g of powder in 100 ml of *n*-hexane for 24 hours. After filtration, the residue was collected and dried to be subjected to further maceration in distilled water at a rate of 5 g per 100 ml of solvent. The filtrate was then collected and dried using a rotary evaporator (Buchi, France). A powder of *F. exasperata* aqueous leaf extract (FEFIX) was obtained with a yield of 14.27 ± 3.26 %. FEFIX was stored at 4 °C until experiments.

Animals

Male Wistar rats weighing 200 and 250 g were used. They were obtained from animal house, Pasteur Institute, Abidjan, Côte d'Ivoire. The animals were grouped and housed in metabolic cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. Prior to the start of the experiment all animals were fasted overnight without water, which was available *ad libitum*. At the end of experiment, rats bloods were sampled from the inferior vena cava after anesthetized them with ether.

Diuretic evaluation

The method described Devane and Ryan [15] was employed, with modification, for the assessment of diuretic activity. The day of the experiment the animals received fluid overload (50 ml/kg bw). Single doses of FEFIX (100 mg/kg bw) and spironolactone (10 mg/kg bw) are then administered orally. A control group received saline 0.9%. The animals were placed individually in metabolic cages where urine is collected and sampled every 2 hours for 24 hours. Urinary excretion was determined from the ratio of the total volume of urine and the volume of fluid overload. The diuretic action is obtained by the ratio of the urinary excretion

of treated group and urinary excretion of the control group. The diuretic activity was determined by the ratio of the diuretic action of test drug and the standard drug.

Electrolytes, creatinine and urea determination

The content of urinary and plasma electrolytes was determined using an automatic analyzer (Hitachi 902, Roche). The determination of sodium and potassium was performed by the technique of photometry. The determination of the chlorine content of the samples, calcium and creatinine was produced by the technique of colorimetry. The content of urea was determined by the principle of kinetics.

Statistical Analysis

Data were expressed as means with standard error of mean (m ± sem) obtained from *n* separate experiments. Statistical analysis of the values and graphical representations of data were performed respectively by GraphPad Instat software (Microsoft, San Diego, California, USA) and GraphPad Prism software (Microsoft, San Diego, California, USA). Differences between the mean statistical validity were assessed through Student-Newman-Keuls multiple comparisons test. The difference between the averages was considered statistically significant at the 5 % ($p < 0.05$).

RESULTS

Urinary excretion

After 24 hours FEFIX and spironolactone increased urine volume (Table 1). The urinary excretion induced by FEFIX was relatively larger than that induced by spironolactone ($p < 0.001$). The diuretic action of FEFIX is also higher than that of spironolactone. The diuretic effect was expressed by FEFIX diuretic activity greater than 1. While the diuretic activity obtained with the control group was less than 1.

Urinary electrolytes

FEFIX and spironolactone (SPLT) increased urinary excretion of electrolytes (Table 2). After 24 hours, the urinary excretions of Na^+ and Cl^- obtained for the two drugs were important. However, the urinary excretions of potassium measured were slightly different from the control ($p > 0.05$). The increase in urinary calcium excretion was observed only with FEFIX. The reports Na^+/K^+ were important to FEFIX and SPLT ($p < 0.001$). Moreover, the ratio obtained with FEFIX was higher than that obtained with the SPLT.

Plasma electrolytes

The aqueous extract of *F. exasperata* leaves (FEFIX) and spironolactone (SPLT) altered plasma electrolyte concentrations (Figure 1). After 24 hours, the drugs studied decreased serum sodium and serum chloride. The serum sodium obtained were 114.09 ± 4.82 mM (FEFIX) and 120.17 ± 4.05 mM (spironolactone). Measured serum chlorine were 67.07 ± 3.62 and 71.17 ± 3.32 mM, respectively for FEFIX and SPLT. Increases in serum potassium and calcium were not significant. FEFIX induced potassium and calcium respectively 4.82 ± 0.41 and 27.92 ± 4.10 mM ($p > 0.05$). SPLT induced serum potassium and calcium respectively 025 ± 4.05 mM and 26.42 ± 5.82 ($p > 0.05$).

Urea and creatinine

Urinary levels of creatinine and urea measured on 24-hour urine showed that FEFIX and SPLT increased urinary excretion of creatinine and urea (Table 3). Urinary creatinine obtained with FEFIX (0.33 mmol) is very significant. The urinary excretion of urea obtained for the two drugs were significant (248.42 ± 17.32 and 203.63 ± 17.98 mmol). Plasma concentrations of creatinine and urea decreased after 24 hours. The decrease in plasma creatinine is only significant for SPLT (0.30 ± 0.04 mM. While the decrease of uremia was significant for both drugs ($p < 0.01$).

Table 1: **Effects of *F. exasperata* aqueous leaf extract (FEFIX) and spironolactone (SPLT) on urine output in rats.**

	Volume of urine excreted (%)	**Diuretic action**	**Diuretic activity**
Saline solution (control)	65.04 ± 5.35	1.00	0.83
SPLT (10 mg/kg b.w.)	82.67 ± 4.29*	1.25	1.00
FEFIX (100 mg/kg b.w.)	120.62 ± 5.42***	1.85	1.53

b.w. : body weight ; n=6 ; m ± sem ; ***** : p<0,05 ; ****** : p<0,01 ; ******* : p<0,001.

Table 2: **Effects of *F. exasperata* aqueous leaf extract (FEFIX) and spironolactone (SPLT) on urinary electrolyte excretion in rats.**

	Urinary electrolyte output (mEq/24 hours)				**Ratio**
	Na^+	K^+	Cl^-	Ca^{++}	Na^+/K^+
Saline solution (control)	5.12 ± 0.30	1.30 ± 0.08	3.52 ± 0.19	2.05 ± 0.15	3.62 ± 0.43
SPLT (10 mg/kg b.w.)	8.81 ± 0.29***	1.22 ± 0.08	5.54 ± 0.18***	2.23 ± 0.13	7.04 ± 0.36***
FEFIX (100 mg/kg b.w.)	13.38 ± 0.31***	1.38 ± 0.10	7.66 ± 0.17***	2.80 ± 0.25*	8.82 ± 0.74***

b.w. : body weight ; n=6 ; m ± sem ; ***** : p<0,05 ; ****** : p<0,01 ; ******* : p<0,001.

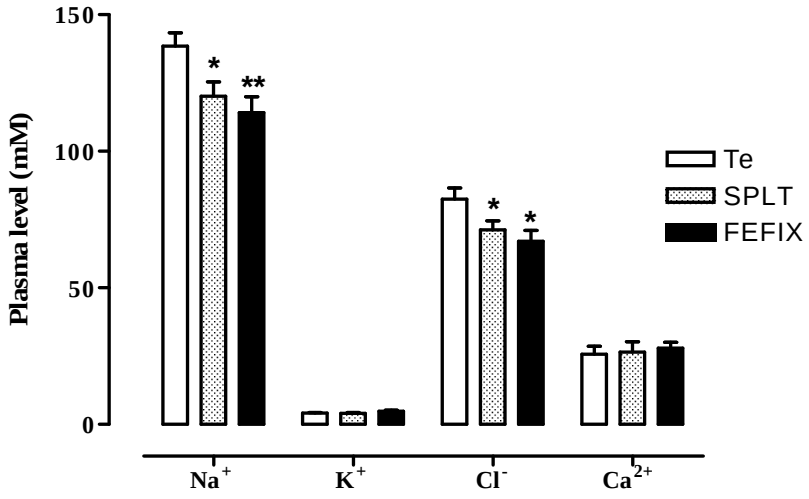

Figure 1: **Plasma concentrations of electrolytes induced by the administration of *F. exasperata* aqueous leaves extract of (FEFIX) and spironolactone (SPLT) after 24 hours in rats. The respective doses of FEFIX and SPLT were 100 and 10 mg/kg b.w., Te: control, * p <0.05, n = 6, m ± sem.**

DISCUSSION

After 24 hours, *F. exasperata* aqueous leaf extract (FEFIX) and spironolactone (SPLT) increased urine volume. The diuretic effect of FEFIX was higher than that observed with SPLT. The emitted urine presented relatively high levels of electrolytes. The diuretic effects of FEFIX resulted from the diversity of chemical compounds present in the extract [16-17]. The phytochemical analysis of the aqueous extract of *Pergularia daemia* revealed the presence of different chemical compounds. This extract increased urine production and urinary excretion of electrolytes [18]. Similar results were also reported the one hand, for the ethanol extract of leaves of *Ricinus communis* [19] on the other hand for different extracts *Laphophytum Leandri* [20]. The effect of a diuretic result of its action on the different part of nephron. This action may cause inhibition of converting enzyme and angiotensin activity of Na/K ATPase like chemical compounds present in the different extracts of *Tropaeolum majus* [21]. The diuretic effects were associated with urinary excretion of electrolytes. FEFIX and SPLT increased the urinary excretion of electrolytes, creatinine and urea. These urinary excretions of electrolytes decreased their plasma concentration. FEFIX Electrolytic effects result of the action of these chemical compounds on ion transporters along the nephron like the aqueous extract of *Amaranthus spinosus*. In fact, this extract increased urinary excretion of electrolytes by inhibiting carbonic anhydrase [22]. In addition, isoquercitine, flavonoid extract from *Tropaeolum majus* increased urinary excretion of sodium and potassium [23]. The chemical

compounds of the aqueous extract of *Hibiscus sabdarifa* increased diuresis and urinary sodium excretion. This effect results from the modulation of the activity of aldosterone [24].

CONCLUSION

After 24 hours, the aqueous extract of *F. exasperata* leaves (FEFIX) increased urine volume and urinary excretion of electrolytes like spironolactone. However, the effects induced by FEFIX are greater than those induced by spironolactone.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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